CHROMATOPHORE CONTROL AND NEUROSECRETION IN THE MUD SHRIMP, UPOGEBIA AFFINIS [1]

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The control of chromatophores in the mud shrimp, *Upogebia affinis,* has not been described. This organism has been used in very few investigations of chromatophores, and in these only as a source of tissues suspected of containing chromatophorotropins. Furthermore, only one account (Hanström, 1937) of the morphology of neuroendocrine structures in this organism is available. In 1948, Hanström summarized the results of his 20-year study of the supraesophageal ganglia and incretory organs in the Malacostraca.

Systematists differ as to whether the tribe Thalassinidea, to which *Upogebia* belongs, should be placed with the Anomura or the Macrura. In 1960, Waterman and Chace included this tribe with the Macrura. On the other hand, in 1961, Green classified the Thalassinidea among the Anomura.

The optic ganglia and sinus glands of the mole crab, *Emerita talpoida,* and of *Upogebia* are closely associated with the supraesophageal ganglia instead of occurring in the eyestalks as is the case in most decapods studied. According to Hanström (1937) the retinal structures and their associated nerve tracts are the only major components of the nervous system present in the eyestalks of these two species. In contrast, the eyestalk of the anomuran, *Pagarus pollicaris,* a hermit crab, contains the usual ganglia, such as the medulla terminalis, and the sinus gland, in addition to the visual structures (Hanström, 1937).

Perkins and Kropp (1932) noted that eyestalks of *Pagurus longicarpus* blanched the shrimp, *Crangon boreas,* which is not an anomuran. In contrast, no response was observed when extracts of eyestalks from *Upogebia* and *Emerita* were injected into the fiddler crab, *Uca,* by Carlson (1936) or into the prawn, *Palaemonetes,* by Hanström (1937). But Hanström did find that eyestalks of *Pagurus pollicaris* blanched eyestalkless *Palaemonetes.* Head extracts of *Upogebia,* on the other hand, darkened eyestalkless *Uca* (Carlson, 1936) and blanched eyestalkless *Palaemonetes* (Hanström, 1937). However, the question remained whether the effect of the head extracts was due to the sinus glands, the supraesophageal ganglia, or both structures, until Sandeen and Baldwin (1962) assayed glands and supraesophageal ganglia of *Upogebia* on *Uca.* Both organs yielded extracts that caused even more melanin dispersion than did extracts of the same tissues from *Uca* when assayed on *Uca.*

In the meantime, Brown and Scudamore (1940) had postulated the presence of

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at least two chromatophorotropins in the sinus gland of *Pagurus pollicaris*. Brown and Saigh (1946) found two antagonistic chromatophorotropins in central nervous organs of *Upogebia affinis, Emerita talpoida, Pagurus pollicaris,* and *P. longicarpus*. Because the assays were performed on *Crangon,* the antagonists in the central nervous organs were termed *Crangon* body-lightening hormone (CBLH) and *Crangon* darkening hormone (CDH).

Through the efforts of several investigators working in the early 1950's (*e.g.,* Passano, 1951; Bliss and Welsh, 1952), the sinus gland was shown to be a storage and release organ, *i.e.,* a neurohemal organ, rather than an actual site of hormone production. Chromatophorotropins appear to be products of neurosecretory cells, transported by axoplasmic flow from the site of formation to neurohemal organs. Miyawaki (1960) is the only investigator who described the cytology of neurosecretory cells in anomurans. He found three types of such cells in the central nervous organs of the crabs *Eupagurus ochotensis* and *Paralithodes brevipes*.

The literature concerning (a) the morphology of neuroendocrine organs and (b) the physiology of chromatophores in typical Macrura is very extensive. In such forms the sinus gland typically resides in the eyestalk (Hanström, 1937). The chromatophore system of Macrura is highly evolved, pigment-concentrating and pigment-dispersing principles having been demonstrated (Fingerman and Aoto, 1962).

The general object of this investigation was to learn the origins and actions of chromatophorotropins in *Upogebia*. The specific aims were to determine (1) the responses of *Upogebia* to extracts of its own eyestalks, sinus glands, and supraesophageal ganglia, and (2) the distribution of neurosecretory cells in the head of *Upogebia*.

Materials and Methods

We are indebted to the personnel from the Supply Department of the Marine Biological Laboratory at Woods Hole, Massachusetts, and to Dr. Muriel I. Sandeen of the Duke Marine Laboratory at Beaufort, North Carolina, for furnishing specimens of *Upogebia affinis*. Specimens used in the bioassays were maintained in the laboratory at Woods Hole in aquaria supplied with constantly flowing sea water.

Red chromatophores on the dorsal surface of the telson and uropods were staged according to the system of Hogben and Slome (1931). Stage 1 represents maximal concentration of the pigment, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. Student's t test was used in the statistical analysis of the data.

Tissue extracts were prepared by grinding the appropriate number of organs in sea water. The concentration was one-third of the organ complement from one mud shrimp per dose, 0.05 ml. Therefore, each dose contained either both eyestalks, both sinus glands, or the supraesophageal ganglia from one mud shrimp.

Paraffin sections of the eyestalks, sinus glands, and supraesophageal ganglia were prepared in the usual fashion. These structures were fixed in (1) Bouin's solution or (2) Helly's solution. Sections 8 and 10 μ thick were stained with (1) Mallory's trichrome, (2) Heidenhain's azan, (3) Gomori's chrome alum hematoxylin-phloxin, or (4) aldehyde fuchsin.

Observations and Results

Responses of erythrophores in Upogebia to tissue extracts

The aim of this experiment was to observe the effects of eyestalks, sinus glands, and supraesophageal ganglia from *Upogebia* on eyestalkless specimens of *Upogebia*. The results are presented in Figure 1 where the results from three experiments (3, 3, and 4 test animals, respectively) are averaged.

Aside from the responses to the extracts, inspection of Figure 1 reveals that the red pigment of eyestalkless *Upogebia* was maximally dispersed before injection of the extracts and sea water. Injection of the tissue extracts caused statistically significant degrees of pigment concentration, $p < 0.001$ for each extract, despite the fact that control injections of sea water evoked some pigment concentration. The chromatophore indexes measured 15 minutes after injection of extracts were used for the statistical calculation. Responses of the chromatophores were highest to extracts of supraesophageal ganglia and least to eyestalk extracts.

Neurosecretory cells in Upogebia

In view of the observation (Fig. 1) that extracts of the supraesophageal ganglia, sinus glands, and eyestalks of *Upogebia* caused a significant concentration of the red pigment in this organism, investigation of these structures for signs of neuro-

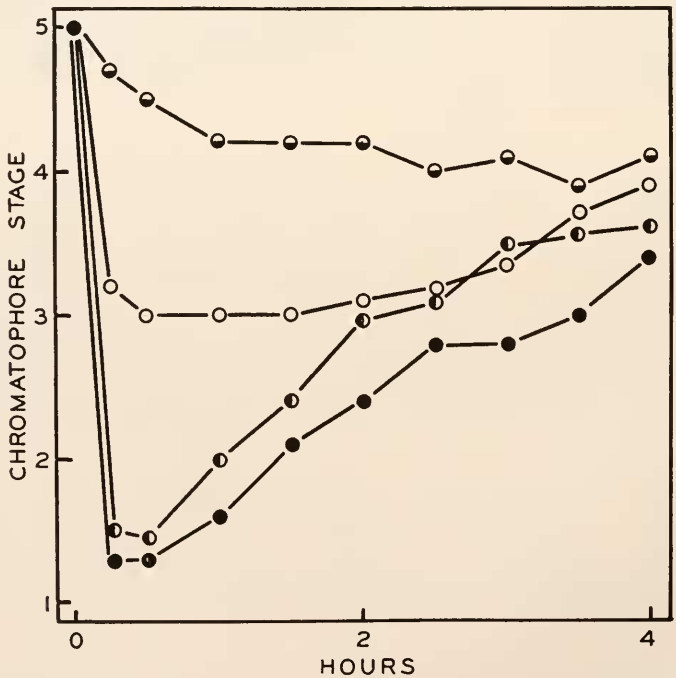

Figure 1. Relationships between chromatophore stage and time following injection of tissue extracts into eyestalkless *Upogebia*. Circles, eyestalks; dots, supraesophageal ganglia; circles half filled on left, sinus gland; circles half filled on bottom, control.

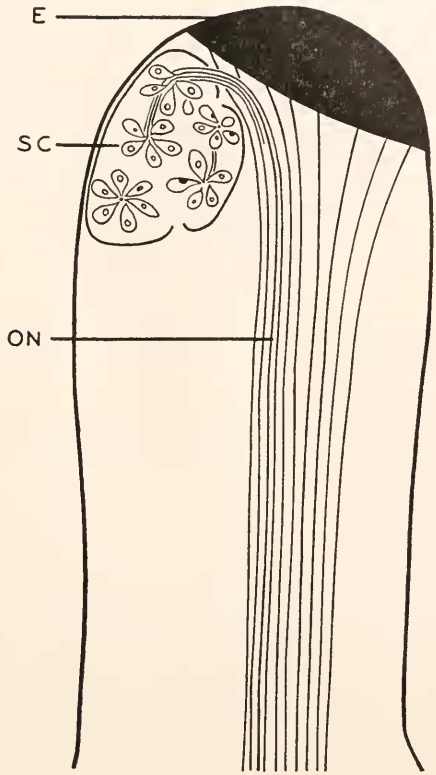

FIGURE 2. Diagram of the eyestalk of *Upogebia*. E, eye; ON, optic nerve; SC, secretory cells.

secretory activity seemed appropriate. Examination of sectional eyestalks revealed a group of 500–700 ovate cells whose cytology and staining properties are typical of neurosecretory cells. This cluster of cells lies in the dorsal half of the distal third of the eyestalk, partially surrounding the optic nerve, and is enclosed by a connective tissue sheath (Fig. 2). The cells are fairly uniform in size, averaging 14 μ wide and 19 μ long, with an axon emerging from one end. The nucleus, which has a conspicuous nucleolus, is centrally located in some of the cells, eccentrically in others. The cytoplasmic granules appear to be neurosecretory products. For example, they stain pink with Heidenhain's azan and are positive to aldehyde fuchsin. In addition to the granules, some of the cells have cytoplasmic vacuoles. The cells are arranged in clusters resembling rosettes (Fig. 3). Because of the unique architecture of this group of cells, the structure will be referred to as the Rosette Body. Blood sinuses were noted in both the central and peripheral portions of the Rosette Body. Pores in the connective tissue sheath afford the blood ready access to these sinuses.

Axons of the optic nerves contained granules that appeared to be neurosecretory products. These granules probably originated in the Rosette Body, inasmuch as the Rosette Body was the only structure observed in the eyestalk that could be the

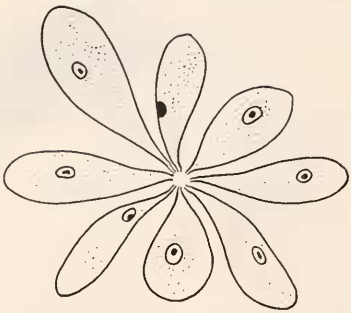

FIGURE 3. Detailed diagram of the cell arrangement in a portion of the Rosette Body.

source of the secretory granules in the optic nerve. The axons from the Rosette Body appear to unite with the optic nerve just proximal to the basement membrane of the eye. Axons of the optic nerve could not be followed after they entered the supraesophageal ganglia.

The general morphology of the sinus glands and supraesophageal ganglia conforms to the description and photograph presented by Hanström (1937). Inspection of histological sections of the supraesophageal ganglia revealed the presence of three distinct types of cells that had neurosecretory staining properties. The largest type has a cell body averaging 24 μ wide and 40 μ long and is restricted to the medullae terminales. The left and right medullae terminales are fused in the midline of the supraesophageal ganglia, as described by Hanström (1937). About six cells of this type occur. They have a round nucleus in the center of the cell body. A conspicuous nucleolus lies near the nuclear membrane. Near the periphery of the cytoplasm occur several vacuoles. The second type, similar in size and shape to the cells that compose the Rosette Body, possesses a large nucleus with a conspicuous nucleolus. Some of these cells are vacuolated. The third type has a round, non-vacuolated cell body 9–11 μ in diameter. This cell is small as far as the usual neurosecretory cell is concerned. Only in the medullae terminales were all three types observed. The sinus gland consisted of nerve endings alone, no cell body having been observed. Of all the ganglia, the medullae terminales had the highest proportion of neurosecretory cells to ganglionic cells.

DISCUSSION

The eyestalks, supraesophageal ganglia, and sinus glands of *Upogebia* possess a principle that concentrates the red pigment in this animal (Fig. 1). As mentioned above, Carlson (1936) and Sandeen and Baldwin (1962) working with *Uca,* and Hanström (1937) working with *Palaemonetes* as assay animals, found that eyestalk extracts of *Upogebia* were ineffective. However, eyestalk extracts of *Upogebia* were effective on *Upogebia* (Fig. 1). The species differences are a possible explanation of the lack of response observed by the other investigators. The greater potency of extracts of supraesophageal ganglia from *Upogebia* compared with sinus gland extracts from this animal (Fig. 1) was also noted by Sandeen and Baldwin (1962) who assayed these extracts on *Uca.*

In *Upogebia* the medulla terminalis X-organ lies in the supraesophageal ganglia. The group of previously undescribed cells that compose the Rosette Body in the eyestalk of *Upogebia* (Figs. 2 and 3) may be homologous with the sensory pore X-organ described by Hanström (1939) in the eyestalk of many crustaceans, *e.g.*, *Palaemon, Crangon,* and *Homarus.* He believed that this organ represents transformed sensory cells of a rudimentary eye papilla or sensory pore. Concentration of the erythrophores in *Upogebia* following injection of the eyestalk extract was presumably due to the secretory product of the Rosette Body. This secretory material may normally be transported by axoplasmic flow into the supraesophageal ganglia and from there to the sinus glands for storage.

Nishida and Miyawaki (1954) described a holocrine gland in the eyestalk of two species of the anomuran *Paralithodes.* In *Paralithodes* the medulla externa, medulla interna, and medulla terminalis occur in the eyestalk but no sinus gland was observed. It may lie on the surface of the supraesophageal ganglia as in *Upogebia.* However, the structure described by Nishida and Miyawaki is not the Rosette Body of *Upogebia.* The present report constitutes the first description of the occurrence of an organ that presumably contains neurosecretory cells in the eyestalks of a crustacean whose optic ganglia do not lie in the eyestalk. Furthermore, the arrangement of the cells is unique for a neurosecretory organ.

As mentioned above, Miyawaki (1962) described three types of neurosecretory cells in the anomurans *Eupagurus ochotensis* and *Paralithodes brevipes.* The widths of the cell bodies were 100–130 μ, 30–60 μ, and 10 μ. In *Upogebia* three sizes of neurosecretory cells occur also. However, the largest type described by Miyawaki is much bigger than any that occurs in *Upogebia.*

The problem of whether *Upogebia* is an anomuran or a macruran should be resolved. The chromatophore system of *Upogebia* does not allow us to decide between these alternatives because so little is known about chromatophore responses of anomurans in general that speculation would be meaningless. On the other hand, the neurosecretory system does offer a clue. The secondary return of the medullae terminales from the eyestalks to the head is typical of anomurans with reduced eyes rather than of macrurans (Hanström, 1948).

SUMMARY AND CONCLUSIONS

1. The eyestalks, sinus glands, and supraesophageal ganglia of the mud shrimp, *Upogebia affinis,* contain a principle that concentrates the pigment in its red chromatophores.

2. A previously undescribed group of cells, with tinctorial properties characteristic of neurosecretory cells, occurs in the eyestalks.

3. Because the arrangement of the cells in this structure is unique among neurosecretory organs, it is proposed that this structure be called the Rosette Body. The secretory product of these cells is probably conveyed through the optic nerve by axoplasmic flow to the supraesophageal ganglia and from there to the sinus glands.

4. The Rosette Body may be homologous with the sensory pore X-organ of higher crustaceans.

5. Three types of cells with tinctorial properties characteristic of neurosecretory cells occur in the supraesophageal ganglia.

6. These observations were discussed in relation to the findings of other investigators.

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